MOLECULAR CLONING, EXPRESSION AND FUNCTIONAL INTERACTION OF p48 SUBUNIT OF CHICKEN CHROMATIN ASSEMBLY FACTOR 1 WITH HISTONE DEACETYLASE 2 AND HISTONE ACETYLTRANSFERASE 1

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ABSTRACT

We cloned and sequenced cDNA encoding p48 subunit of the chicken CAF-1, chCAF-1p48, and histone acetyltransferase-1, chHAT-1 from chicken DT40 cell lines. We showed that the p48 subunit of CAF-1 tightly binds to two regions of chicken histone deacetylase 2, chHDAC-2, located between amino acid residues 82-180 and 245-314, respectively. We also established that two N-terminal, two C-terminal, or one N-terminal and one C-terminal WD repeat motif of chCAF-1p48 are required for this interaction. The GST pulldown assay, involving truncated and missense mutants of chCAF-1p48, revealed not only that a region containing the seventh WD dipeptide motif of chCAF-1p48, comprising amino acids 376-405, binds to chHAT-1 in vitro, but also that mutation of the motif has no influence on the in vitro interaction. We also established that the region, which is located between amino acids 380-408 of chHAT-1 and contains a leucine zipper motif, is required for its in vitro interaction with chCAF-1p48. Mutation on each of four Leu residues in the leucine zipper motif of chHAT-1 causes the disappearance of the interaction with chCAF-1p48. These results should be useful information for understanding the participation of chCAF-1p48 protein as histones chaperone in DNA-utilizing processes, such as replication, recombination, repair and gene expression in DT40 chicken B cell.

Keywords: *Chromatin assembly factor-1; histone deacetylase 2; histone acetyltransferase 1; polymerase chain reaction; polyacrylamide gel electrophoresis*

ABSTRAK

Telah dilakukan penelitian yang bertujuan mengkloning dan menentukan urutan cDNA dari gen yang menyandi subunit p48 dari CAF-1 (CAF-1p48), histone deasetilase-2 (HDAC-2), dan histone asetil transferase-1 (HAT-1) pada sel ayam DT40. Hasil penelitian menunjukkan bahwa subunit p48 dari CAF-1 dapat berinteraksi dengan histone deasetilse-2 pada dua lokasi yaitu masing-masing antara residu asam amino 82-180 dan 245-314. Sebaliknya ada dua lokasi pada N-terminal, dua lokasi pada C-terminal atau masing-masing satu lokasi pada N-terminal dan satu lokasi pada C-terminal dari CAF-1p48 diperlukan untuk berinteraksi dengan histone deasetilase-2. Dengan teknik GST pulldown assay, tidak hanya menemukan bahwa lokasi ke tujuh dari motif WD dipeptida antara residu asam amino 376-405 dari CAF-1p48 dapat berinteraksi dengan HAT-1, tetapi mutasi pada motif tersebut tidak mempengaruhi interaksi kedua jenis protein. Pada residu asam amino 380-408 dari HAT-1 terdapat leucine zipper motif yang esensial untuk berinteraksi dengan CAF-1p48. hasil penelitian ini dapat memberikan informasi untuk memahami peranan protein CAF-1p48 dalam berbagai aktivitas molekul DNA seperti proses replikasi, rekombinasi, dan ekspresi gen dalam sel ayam DT40.

Kata Kunci: *Chromatin assembly factor-1; histone deasetilase-2; histone asetiltransferase 1; reaksi polimerisasi berantai; elektroforesis gel poliakrilamida*

INTRODUCTION

In the nucleus of the eukaryotes cells, DNA is packaged into a nucleoprotein structure known as chromatin. The basic repeating unit of chromatin, the nucleosome, consists of approximately two turns of DNA wrapped around an octamer of core histone proteins [1]. Understanding of the mechanisms of chromatin assembly and alterations in the chromatin structure in eukaryotes is a fundamental goal, because they have been thought to be predominantly involved in DNA-utilizing processes, such as replication, recombination, repair and gene expression.

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